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Biodiversity of arbuscular mycorrhizal colonization and spore population in different agroforestry trees and crop species growing in Dinajpur, Bangladesh

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Abstract: Biodiversity of arbuscular mycorrhizal colonization and spore population was investigated in different agroforestry trees and crop species collected from different locations of Dinajpur district of Bangladesh. Roots and rhizosphere soils of *Albizia procera* Benth., *Capsicum frutescens* L, *Curcuma domestica* Vahl., *Dalbergia sissoo* Roxb. and *Swietenia macrophylla* King. from Dashmail; *C. domestica*, *D. sissoo*, *Eucalyptus camaldulensis* Dehnn., *Gmelina arborea* (Roxb) DC and *Oryza sativa* L. from Kantaji and *C. domestica*, *D. sissoo*, *Litchi chinensis* Sonn. and *O. sativa* from Ramsagar were collected. Roots and soils were assessed following standard methods. The range of AM colonization was recorded 36%–79% from Dashmail. The highest AM colonization was recorded in *C. frutescens* (79%) and the lowest was in *C. domestica* (36%). The range of colonization was recorded as 33%–70% from Kantaji. The highest AM colonization was recorded in *G. arborea* (70%) and the lowest was in *O. sativa* (33%). The range of AM colonization was recorded as 35%–70% from Ramsagar. The highest AM colonization was recorded in *D. sissoo* (70%) and the lowest was in O. sativa (35%). Arbuscular mycorrhizal spore population varied from 54 to 140/100g dry soil in the soils from Dashmail. The highest was in the soils of *D. sissoo* (140) and the lowest was in C. domestica (54). The spore population varied from 63 to 221 in Kantaji. The highest was in *G. arborea* (221) and the lowest was in O. sativa (63). The range population in Ramsagar varied from 69 to 160. The highest was recorded in *D. sissoo* (160) and the lowest was in *L. chinensis* (69). No significant relationship of soil pH and soil OM with AM colonization and with spore population was observed. Simpson's index of diversity (Ds) and Shannon's index of diversity- (Hs) were highest in the soil of *D. sissoo* from Kantaji and the lowest in the soils of *O. sativa* from Ramsagar. Biodiversity of AM colonization, spore population and the distribution of AM fungi in the rhizosphere soils of different agroforestry plants indicated the occurrence of AM fungi, mycotrophic nature of the trees and crop species, contribution and necessity of AM fungi and the AM dependence of the agroforestry plants growing in Dinajpur district of Bangladesh.

Keywords: Biodiversity; Agroforestry; Mycorrhiza; Colonization; Spore population.

Introduction

Agroforestry has become popular in Asia including Bangladesh as a means of agricultural and rural development (Bentley 1993). Different public programs like social forestry, community forestry or participatory forestry have encouraged the spread of agroforestry systems as part of the development of sustainable production systems for rural communities. Different government and nongovernment organizations undertook agroforestry initiatives to boost up the rural economy of Bangladesh through Village and Farm Forestry Project (VFFP) in different parts of Dinajpur (Ahmed 2001). Dinajpur district consists of plio-pleistocene terrace soils in the 'Barind Tract' region of Bangladesh (Hassan 1999). Deficiency of major nutrients, acidic nature of the soil, draught etc are the main constraints in the agroforestry systems of this area. Low rainfall and deep groundwater level make irrigation difficult and crop-loss occurs due to the draught during summer or flood during rainy season (Ahmed 2001). Shortage of organic matter in the soil is also a limiting factor in the agroforestry regions. Continuous removal and burning of crop residues from the agroforestry fields might cause these problems.

In low fertile soils including agroforestry systems like Dinajpur, mycorrhizal symbiosis has great potential to reduce production-cost improving plant growth by taking up P and other micronutrients, controlling soil borne plant diseases, improving water balance and reducing drought stress (see Dhar and Mridha 2003). AM fungi play a crucial role in facilitating microbial and plant functions. The potential of AM fungi to mediate interplant nutrient exchange (Francis and Read 1984; van Kessel *et al.* 1985) is of particular importance. However, in the description of agroforestry systems arbuscular mycorrhizal fungi are not often considered. In Bangladesh, information on the biodiversity of arbuscular mycorrhizal fungal colonization in different agroforestry plants are very little (Dhar and Mridha 2003; Mridha and Rahman 2001). In the present study, survey was conducted to observe the biodiversity of arbuscular mycorrhizal fungal colonization in the roots and the population of arbuscular mycorrhizal fungi in the rhizosphere soils of different agroforestry trees

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and crops growing in different areas of Dinajpur district of Bangladesh.

Materials and methods

Site selection and sample collection

This investigation on the biodiversity of arbuscular mycorrhizal colonization and spore population in different agroforestry plant species was conducted in the north-western part of Bangladesh. The study sites are located in different areas (Dashmail, Kantaji and Ramsagar) of Dinajpur district. Three replicated samples of roots and rhizosphere soil of *Albizia procera* Benth., *Capsicum frutescens* L, *Curcuma domestica* Vahl., *Dalbergia sissoo* Roxb. and *Swietenia macrophylla* King. from Dashmail; *C. domestica*, *D. sissoo*, *Eucalyptus camaldulensis* Dehnn., *Gmelina arborea* (Roxb) DC and *Oryza sativa* L. from Kantaji and *C. domestica*, *D. sissoo*, *Litchi chinensis* Sonn. and *O. sativa* from Ramsagar were collected from 0-15 cm depth.

Determination of arbuscular mycorrhizal colonization

Roots were cleared and preserved in 5% formalin and soils were studied immediately for spore population to avoid any damage and desiccation of the spores. Spores were extracted by wet sieving and decanting method (Gerdemann and Nicolson, 1963). On the Whatman No.1 filter paper, spores were spread with water, on which squares were drawn with intersected grid lines for easy counting. Morphologically similar spores were picked up with the help of soft forceps and mounted in the Melzer's reagent and PVLG. The AM fungal spores were identified up to genus level (Schenck and Perez 1990). Percent population of individual genus was calculated (Dhar and Mridha, 2003). Roots were stained in aniline blue following standard method (Phillips and Hayman 1970) with some modifications.

Total percent AM colonization, percent colonization of different AM fungal structures (mycelium, vesicles and arbuscules) and intensity of AM structural colonization were calculated. Mycelial colonization was considered as total AM colonization and the intensity of structural colonization was recorded as poor, moderate and abundant (Dhar and Mridha 2003). Soil organic matter (OM) and pH were recorded (Chowdhury *et al.* 1969). Simpson's diversity index (Ds) and Shannon's diversity index (Hs) were calculated (see Dhar and Mridha 2006).

Results

Arbuscular mycorrhizal colonization

The data on total AM colonization in different agroforestry trees and crop species from Dashmail, Kantaji and Ramsagar are presented in the Figs. 1, 2 & 3. The ranges of AM colonization were 36%–79% in Dashmail, 33%–70% in Kantaji and 35%–70% in Ramsagar. The highest AM colonization was with *C. frutescens* (79%) in Dashmail, *G. arborea* (70%) in Kantaji and *C. domestica* (70%) in Ramsagar. The lowest AM colonization was with *D. sissoo* (36%) in Dashmail, *O. sativa* (33%) in Kantaji and *L. chinensis* (35%) in Ramsagar. Arbuscular mycorrhizal structural colonization and intensity of colonization in different agroforestry plants from Dashmail are presented in the Table 1. Total AM structural colonization varied significantly as indicated by DMRT at $P<0.05$. The ranges of total mycelial, vesicular and arbuscular colonization were recorded 36%–79%, 26%–54% and 27%–57% respectively. Poor, moderate and abundant intensity were recorded 18%–54%, 42%–65% and 5%–19% for mycelial colonization; 25%–94%, 6%–62% and 7%–13% for vesicular colonization and 50%–77%, 17%–38% and 5%–33% for arbuscular colonization respectively. Arbuscular mycorrhizal structural colonization and intensity of AM structural colonization in different agroforestry plants from Kantaji are presented in the Table 2. The variation in the percent colonization of different AM structures in Kantaji was significant as showed by the DMRT at $P<0.05$. Mycelial, vesicular and arbuscular colonization ranged 33%–70%, 21%–57% and 14%–78% respectively. The ranges of poor, moderate and abundant intensity were 24%–78%, 14%–43% and 4%–33% for mycelial colonization; 12%–86%, 9%–51% and 6%–37% for vesicular colonization and 26%–38%, 46%–67% and 11%–20% for arbuscular colonization. Arbuscular mycorrhizal structural colonization and intensity of AM structural colonization in different agroforestry plants from Ramsagar are presented in the Table 3. In Ramsagar, the ranges of mycelial, vesicular and arbuscular colonization varied significantly. Mycelial, vesicular and arbuscular colonization ranged 35%–70%, 18%–44% and 22%–40% respectively. The ranges of poor, moderate and abundant intensity were 43%–65%, 30%–57% and 5% for mycelial colonization; 50%–86%, 9%–25% and 8%–25% for vesicular colonization and 57%–100%, 29%–33% and 8%–10% for arbuscular colonization respectively.

Arbuscular mycorrhizal spore population

Population density of arbuscular mycorrhizal (AM) fungal spores in the rhizosphere soil of different locations showed significant variation (Table 4). Total population of AM fungal spores was recorded 54–140/100g dry soil in Dashmail, 63–221 in Kantaji and 69-160 in Ramsagar. The highest was recorded with *D. sissoo* (140) and the lowest was with *C. domestica* in Dashmail (54). Percent population of *Glomus, Sclerocystis, Acaulospora*, *Entrophospora*, *Gigaspora* and *Scutellospora* were recorded 72–88%, 2%–9%, 3%–11%, 0.00%, 6%–10% and 3% from Dashmail. In Kantaji, the highest AM spore population was recorded with *E. camaldulensis* (221) and the lowest was with *O. sativa* (63). *Glomus, Sclerocystis, Acaulospora*, *Entrophospora*, *Gigaspora* and *Scutellospora* were recorded 55–90%, 6%–10%, 5%–7%, 3%–20%, 6%–10%, 4%–10% in Kantaji. In Ramsagar, the highest spore population was recorded with *C. domestica* (160) and the lowest was with *L. chinensis* (69). *Glomus, Sclerocystis, Acaulospora*, *Entrophospora*, *Gigaspora* and *Scutellospora* were recorded 45%–92%, 20%, 5%–13%, 3%–19%, 4%–9% and 16% from Ramsagar respectively.

The range of pH and organic matter recorded in Dashmail, Kantaji and Ramsagar have been presented in the Table 5. Though the R^2 value showed positive relation between pH and AM colonization and OM and AM colonization in Ramsagar, no definite and significant relations were observed between pH and AM colonization, pH and AM spore population, OM and AM colonization and OM and spore population in Dashmail and Kantaji (Table 6). In Dashmail, Simpson's diversity index (Ds) and Shannon's diversity index (Hs) were calculated highest with *C. domestica* followed by *D. sissoo*, *S. macrophylla* and *C. frutescens*. The lowests were with *A. procera* (Fig 4). The highest diversity indices were in *D. sissoo* followed by *G. arborea, O.*

sativa, and *E. camaldulensis* in Kantaji (Fig. 5). The lowests were with *C. domestica* in Kantaji. In Ramsagar, the highest diversity indices were in *L. chinensis* followed by *O. sativa* and *D. sissoo*. The lowests were *C. domestica* in Ramsagar (Fig. 6).

Table 1. Intensity of arbuscular mycorrhizal structural colonization in different agroforestry trees and crop species from Dashmail, Dinajpur

Plant species	Total colonization (%)			Intensity of structural colonization (%)								
	Mycelium	Vesicles	Arbuscules	Mycelium			Vesicles			Arbuscules		
				P*	M	A	P	M	A	P	M	A
Albizia procera	57c**	26d	36b	18	65	17	54	33	13	50	30	20
Capsicum frutescens	79a	35c	27d	36	45	19	25	62	13	50	17	33
Curcuma domestica	36e	26d	21e	54	40	5	94	6	--	77	18	5
Dalbergia sissoo	65b	38b	57a	33	53	14	62	31	7	50	38	12
Swietenia macrophylla	55d	54a	31c	45	42	13	55	37	8	71	16	13

*P-Poor, M, Moderate, A-Abundant; ** Different letters showed significant variations as indicated by DMRT at P<0.05.

Table 2. Intensity of arbuscular mycorrhizal colonization in different agroforestry trees and crop species from Kantaji, Dinajpur.

Plant species	Total colonization (%)			Intensity of structural colonization (%)								
	Mycelium	Vesicles	Arbuscules	Mycelium			Vesicles			Arbuscules		
				P*	M	A	P	M	A	P	M	A
Curcuma domestica	52b**	45b	38b	76	20	4	85	9	6	36	52	12
Dalbergia sissoo	48c	25c	21d	26	42	32	12	51	37	26	54	20
Eucalyptus camaldulensis	48c	46b	27c	40	40	20	42	42	16	33	67	--
Gmelina arborea	70a	57a	78a	24	43	33	44	39	17	38	46	16
Oryza sativa	33d	21d	14e	78	14	8	86	14	--	34	55	11

*P-Poor, M, Moderate, A-Abundant; ** Different letters showed significant variations as indicated by DMRT at P<0.05.

Table 3. Intensity of arbuscular mycorrhizal coloizatin in different agroforesty trees and crop species from Ramsagar, Dinajpur.

Plant species	Total colonization (%)			Intensity of structural colonization (%)								
	Mycelium	Vesicles	Arbuscules	Mycelium			Vesicles			Arbuscules		
				P*	M	A	P	M	A	P	M	A
Curcuma domestica	70a**	44a	40a	65	30	5	83	9	8	63	29	8
Dalbergia sissoo	63b	27b	23c	43	57	--	50	25	25	57	33	10
Litchi chinensis	35d	24c	32b	60	40	--	86	14	--	100	--	--
Oryza sativa	48c	18d	22c	64	36	--	80	20	--	100	--	--

*P-Poor, M, Moderate, A-Abundant; ** Different letters showed significant variations as indicated by DMRT at P<0.05.

Table 4. Biodiversity of am fungi and their distribution in the rhizosphere soils of different agroforestry trees and crop species from different locations of Dinajpur.

Location	Crops and tree species	Total spore population	Glm * (%)	Scl. (%)	Acl (%)	Ent (%)	Gig. (%)	Scut. (%)
Dashmail	*Albizia procera*	103d**	88	2	--	--	10	--
	Capsicum frutescens	129b	76	5	11	--	8	--
	Dalbergia sissoo	140a	72	9	10	--	9	--
	Curcuma domestica	54e	83	4	--	--	6	--
	Swietenia macrophylla	121c	87	7	3	--	--	3
Kantaji	*Curcuma domestica*	111d	90	6	--	--	--	4
	Dalbergia sissoo	153b	84	--	--	10	--	6
	Gmelina arborea	121c	55	10	5	20	--	10
	Eucalyptus camaldulensis	221a	87	--	--	3	10	--
	Oryza sativa	63e	83	--	7	4	6	--
Ramsagar	*Curcuma domestica*	160a	86	--	--	5	9	--
	Dalbergia sissoo	69d	45	20	--	19	--	16
	Litchi chinensis	116b	83	--	13	--	4	--
	Oryza sativa	84c	92	--	5	3	--	--

* Glm-*Glomus*, Scl- *Sclerocystis*, Acl- *Acaulospora*, Ent-*Entrophospora*, Gig-*Gigaspora*, Scut-*Scutellospora*

** Different letters showed significant variations as indicated by DMRT at P<0.05.

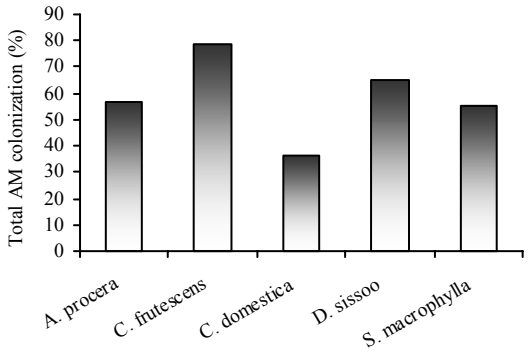

Fig. 1 Total AM colonization (%) in the roots of differentagroforestry trees and crops growing in Dashmail, Dinajpur

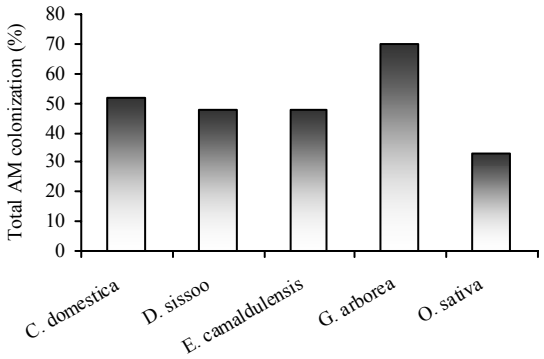

Fig. 2 Total AM colonization (%) in the roots of different agroforestry trees and crops growing in Kantaji, Dinajpur

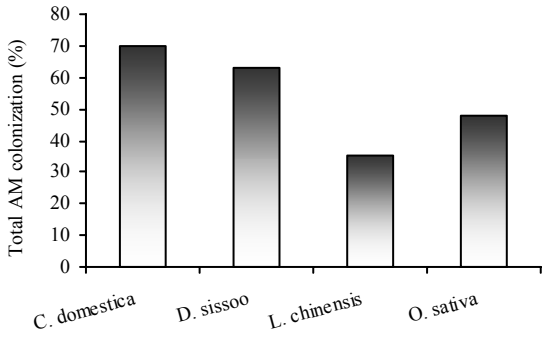

Fig. 3 Total AM colonization (%) in the roots of different agroforestry trees and crops growing in Ramsagar, Dinajpur

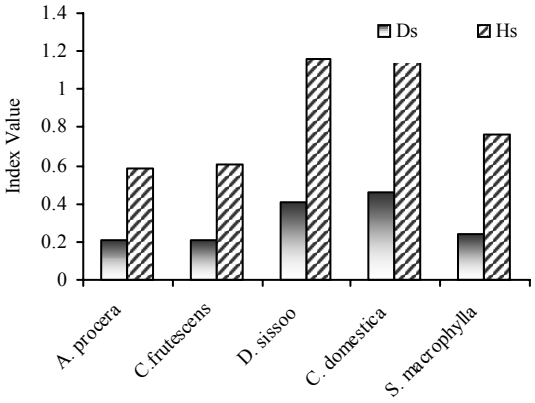

Fig. 4 Diversity Index of AM fungi in the rhizosphere soil of different agroforestry crop species from Dashmail, Dinajpur.

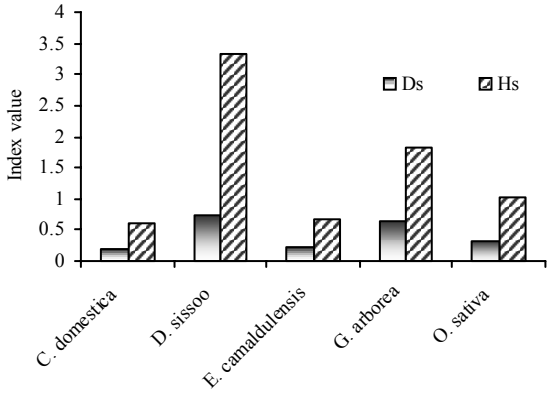

Fig. 5 Diversity indices of AM fungi in the rhizosphere soil of different agroforestry plants growing in Kantaji, Dinajpur

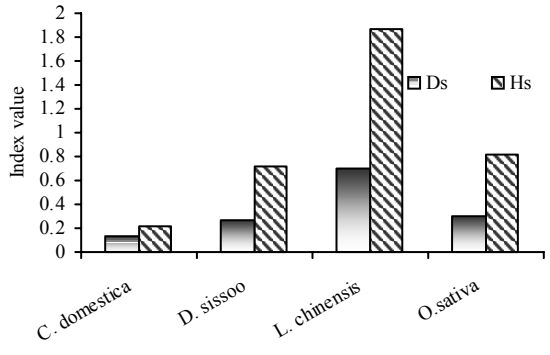

Fig. 6 Diversity Index of AM fungi in the rhizosphere soil of different agroforestry plants growing in Ramsagar, Dinajpur

Discussion

Different agroforestry plants under study were found mycorrhizal. Significant variations ($P<0.05$) in arbuscular mycorrhizal colonization and spore population in rhizosphere soils was observed in different plants irrespective of the locations. This study confirms the widespread occurrence of arbuscular mycorrhizal fungi in the agroforestry soil of different locations of Dinajpur district. The findings were in consistent with the reports of many authors (Pande and Tarafder, 2004; Dhar and Mridha 2003). Different edapho-climatic factors and environmental factors like

soil type, soil quality, low nutrient status of soil, high aeration, soil pH, organic matter, soil moisture, rainfall, temperature, etc might be responsible to the variation in root colonization and spore population (Sharma *et al.* 1986). Different gradients of soil factors and the strong effects of plant factors on the mycorrhiza formation, mycorrhizal function and adaptation of the mycorrhizal fungi to the respective soil conditions might be responsible. These factors of Dinajpur agroforestry systems might be variable in different locations and the variations in AM colonization and spore population could be attributed as such. Moisture might also be important to the arbuscular intensity (O'Connor *et. al*. 2001).

Table 5. Range of ph and organic matter in the rhizosphere soils of different agroforestry trees and crop species growing in different locations of dinajpur

Locations	Plant species	pH	OM (%)
Dashmail	*Albizia procera*	4.48	2.61
	Capsicum frutescens	4.51	4.52
	Dalbergia sissoo	4.22	3.84
	Curcuma domestica	4.62	2.35
	Swietenia domestica	4.76	3.54
Kantaji	*Curcuma domestica*	4.74	3.56
	Dalbergia sissoo	4.44	2.89
	Eucalyptus camaldulensis	4.90	3.41
	Gmelina arborea	4.42	3.11
	Oryza sativa	4.48	3.66
Ramsagar	*Curcuma domestica*	4.59	2.12
	Dalbergia sissoo	4.61	3.89
	Litchi chinensis	4.57	3.02
	Oryza sativa	4.87	3.76

Table 6. Correlations between ph, om, am colonization and am fungal spore population in the rhizosphere soil of different trees and crop species growing in different locations of Dinajpur.

Locations	Correlations between pH and AM colonization (R^2)	Correlations between pH and AM spore population (R^2)	Correlations between OM (%) and AM colonization (R^2)	Correlations between OM (%) and AM spore population (R^2)
Dashmail	0.02	0.03	0.03	0.03
Kantaji	0.04	0.01	0.04	0.01
Ramsagar	0.60	0.34	0.60	0.34

Disturbances like soil erosion, tillage, fertilizer and pesticide application, weeding and crop rotations are the common practices in the agroforestry systems of Dinajpur that might affect the diversity of AM fungi in several ways. Destruction of extra radical hyphae by disturbing agents diminishes mycorrhizal inocula and decreases the AM colonization by disrupting the AM fungal hyphal network in soil. Once the equilibrium is disturbed, the population dynamics of AM fungi are disrupted and biasness can be developed towards a few particular or even one dominant fungus (Pandey and Tarafder 2004; Vivekanandan and Fixen 1991).

The variations in AM colonization under present study might explain that the plant species regarding mycorrhizal colonization had a narrow to broad range of colonization. Variable susceptibility of different agroforestry plants , seasonal variation in development of host plants, host efficiency in soil resource capture and utilization might have the potential to the variation in root colonization and spore population(see Dhar and Mridha 2003). Host phenology, root phenology and root production are related to the patterns of spore production, spore germination, hyphal branching etc (Brundrett 2002) and thus cause varied mycorrhizal colonization. Diverse type of AM fungi in the rhizosphere soils of individual plant species and seasonal sporulation of AM fungi might also cause the variation (Sharma *et al.* 1986). No significant relationship was observed between mycelial colonization and spore population. Many researchers found positive relationship between AM colonization and spore population (Louis and Lim 1987) whiles others found negative relationship (Fontenla *et al.* 1998).

Glomus and *Acaulospora* were common and widely distributed genera among the samples. Dominancy of *Glomus* in the present study is in agreement with the reports of Sharma *et al.* (1986) and Pande and Tarafder (2004). The predominance of *Glomus* under varying soil conditions may be due to the fact that they are widely adaptable to the varied soil conditions and can survive in acidic as well as in alkaline soils (Pande and Tarafder 2004). The distribution of AM fungi can be measured in terms of fungal species occurring under certain conditions (Sieverding, 1991). Variations of diversity indices (Ds, Hs) observed in the present study might be due to the climatic factors, different life durations of the host plants, different disturbing agents etc which might be responsible for spore abundance and distribution of AM fungi (Chaurasia *et al.* 2005; Muthukumar and Udaiyan, 2000). Diversity of mycorrhizal fungi might often be variable with the same plant (Allen and Boosalis 1983). The varied soil pH of different locations of Dinajpur areas could play vital role in determining the diversity and the distribution of different AM fungi in different locations (Abbott and Robson, 1991). However, no significant relationship of the soil parameters with AM colonization and spore population was observed in the present study. Pandey and Tarafder (2004) did not mention any relationship of pH with AM colonization and spore population in the agroforestry systems of Rajasthan.

To maintain optimum diversity, agronomic practices need to be selected carefully and rational management of AM population is necessary at both high and low levels of fungal diversity. More studies are needed regarding the management and conservation of indigenous AM fungal strains in the agroforestry systems of Dinajpur district to make the farmers and people aware of the potential of mycorrhiza to sustainable management of the agro-ecosystems in a sound environment.

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